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Thymus Mast Cell Numbers Following Perinatal and Adult Exposures to Low Intensity 0.5 Hz Magnetic Fields*

by
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ABSTRACT. — In a factorial design, 40 male 200-day-old rats that had been exposed from 2.5 days before to 2.5 days after birth to either 0.5 Hz rotating magnetic fields (RMFs) between 10^{-3}T to 10^{-6}T or to sham fields and maintained after weaning in one of two typical caging conditions were exposed as adults to either one of three 0.5 Hz RMF intensities (10^{-6}T, 10^{-7}T or 10^{-8}T) or to sham fields or to colony room control conditions. The numbers of mast cells (MCs/mm^2) were determined for thymus tissues stained with thionin and toluidine blue. Thymuses from adult rats that had been perinatally exposed to the RMF displayed a marginally significant 20% to 35% elevation in MC numbers relative to sham-field controls. However the adult exposures did not significantly affect the MC numbers. The two postweaning caging conditions, a non-magnetic field comparator variable, induced a significant 35% difference in MC numbers. The absence of significant perinatal by adult RMF exposure interactions indicated that early magnetic field exposure did not alter adult thymus responsivity to weaker but more natural intensity levels.

INTRODUCTION

The thymus seems to be the principal source of mast cells (MCs) in the rat body (Csaba, 1972). These cells contain histamine, heparin, serotonin, proteinpolysaccharides and basic protein and have been implicated strongly in allergic reactions, localized occurrences of edemas and erythema (Mikolajczyk, 1963) and the induction of "restless" motor activity. Intense thymic masticytopoiesis can be induced in the rat by stimuli like pregnancy, X-rays, tumorous conditions, colchicine administration and cortisone injections (Csaba, 1972). Simple administration of 2 mA of galvanic current for 4.5 min can induce significant elevations of thymic mast cells within 24 h (Csaba, Horvath and Horvath, 1961). These data suggest that mast cell numbers in rat thymus may be a sensitive measure of weak environmental stimuli such as low intensity, extremely low frequency electromagnetic fields.

Experiments by Persinger, Carrey and Lafrenière (1978) have demonstrated that significant reductions in thymus weights occur in adult rats following perinatal exposure to 0.5 Hz rotating magnetic fields (RMF). Although we have not found significant thymus weight changes in rats exposed as adults to these fields, other experimenters

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have reported ELF associated alterations in thymic-related immunological reactions (see Persinger, 1974). The present study was designed to answer three specific questions: (1) Do 5-day exposures of adult rats to variation intensities (10^{-6}T to $<10^{-9}$T) similar to natural sources alter thymic MC numbers? (2) Do perinatal 0.5 Hz RMF exposure produce long term alterations in thymic MC numbers and (3) Do perinatal exposures to RMFs at intensities known to alter thymus weights alter this organ's MC population during adulthood to lower, more natural exposure intensities?

The existence of any statistically significant effect associated with low level magnetic field exposures must be evaluated carefully with regards to its absolute magnitude and practical relevance. The former problem can be alleviated somewhat by incorporating other, non-magnetic variables known to influence rat physiology/behavior, into the experimental design. Consequently, in a factorial design, we selected 5 adult conditions (4 field intensities and 1 colony control), 2 perinatal magnetic field conditions and 2 postweaning caging conditions to determine the responsiveness of thymic MC numbers.

METHOD

SUBJECTS. — Forty (40), 180 to 210 day old naive male albino Wistar rats selected from 18 litters that had been exposed perinatally for 5 days to either: 10^{-3}T, 10^{-4}T, or 5 x 10^{-6}T variation 0.5 Hz rotating magnetic fields (RMF) or to one of three sham-field conditions, were used as subjects. Their parents had been obtained from Bio-Breeding Laboratories of Canada, Limited.

PROCEDURE. — In 6 separate exposure periods, a total of 18 pregnant 90- to 120-day-old females were exposed to one of three separate 0.5 Hz RMF variation intensity areas: 10^{-3}T, 10^{-4}T, or 5 x 10^{-6}T or to one of three sham fields which involved the same areas used in the RMF sequences only the magnets had been removed (see Persinger, Lafrenière and Ossenkopp, 1974 for diagram of apparatus). All females were exposed in single 28 x 18 x 12 cm plastic cages to the experimental room, for either the RMF or sham-field conditions, on day 19 of gestation, (about 2.5 days before birth). Within 24 h of birth, the litters were reduced to 8 pups. The following experimental room parameters existed: (1) continuous and indirect lighting from overhead fluorescent lamps, 45 ± 5 lx at floor of cages; (2) masking sound from continuous operating motor, 70 db; and (3) (thermostat controlled ambient temperature) 24.5 ± 0.2°C, measured beside cages. At the end of the (perinatal) exposure period, the litters and mothers were removed to the colony room (LD 12:12, ambient temperature 22 ± 1°C, background noise 70 ± 2 db) and placed in 46 x 20 x 14 cm cages containing 6 mm corncob bedding After weaning, the rats were separated according to sex and placed in groups of three in either standard wire cages (40.5 x 24 x 18 cm) or in plastic cages (46 x 20 x 14 cm); the corncob bedding in the latter was changed twice per week. The positions of the cages containing the perinatal RMF-exposed and sham field rats were counterbalanced in the rack or shelf space to attenuate possible position artifacts.

During 8, separate experimental runs between 9 May to 27 June 1976, the 200 ± 5-day-old subjects were taken to the experimental room and exposed continuously for 5 days to 0.5 Hz RMFs of either 10^{-6}T or 10^{-7}T, or to 10^{-8}T or sham field (less than 10^{-9}T) variations; the latter condition was associated with essentially no variation to the nearest 1γ on a McPhar magnetometer. The first pair of intensities was produced by a pair of weak iron horseshoe magnets (IM) while the second pair of intensities was produced by aluminum "magnets" (AM) which when attached to the output shafts allowed for the small inductions (10^{-8}T or less than 10^{-9}T) in the exposure areas. Both sets of magnets were balanced to the nearest g to avoid possible weight artifacts. The pairs of magnets were used in the following experimental order: IM, AM, IM, AM, AM, IM, AM, IM; rats that had been housed in the plastic cages were used in series 1, 2, 7, and 8 while those housed in the wire cages were used in series 3 to 6. In a given exposure

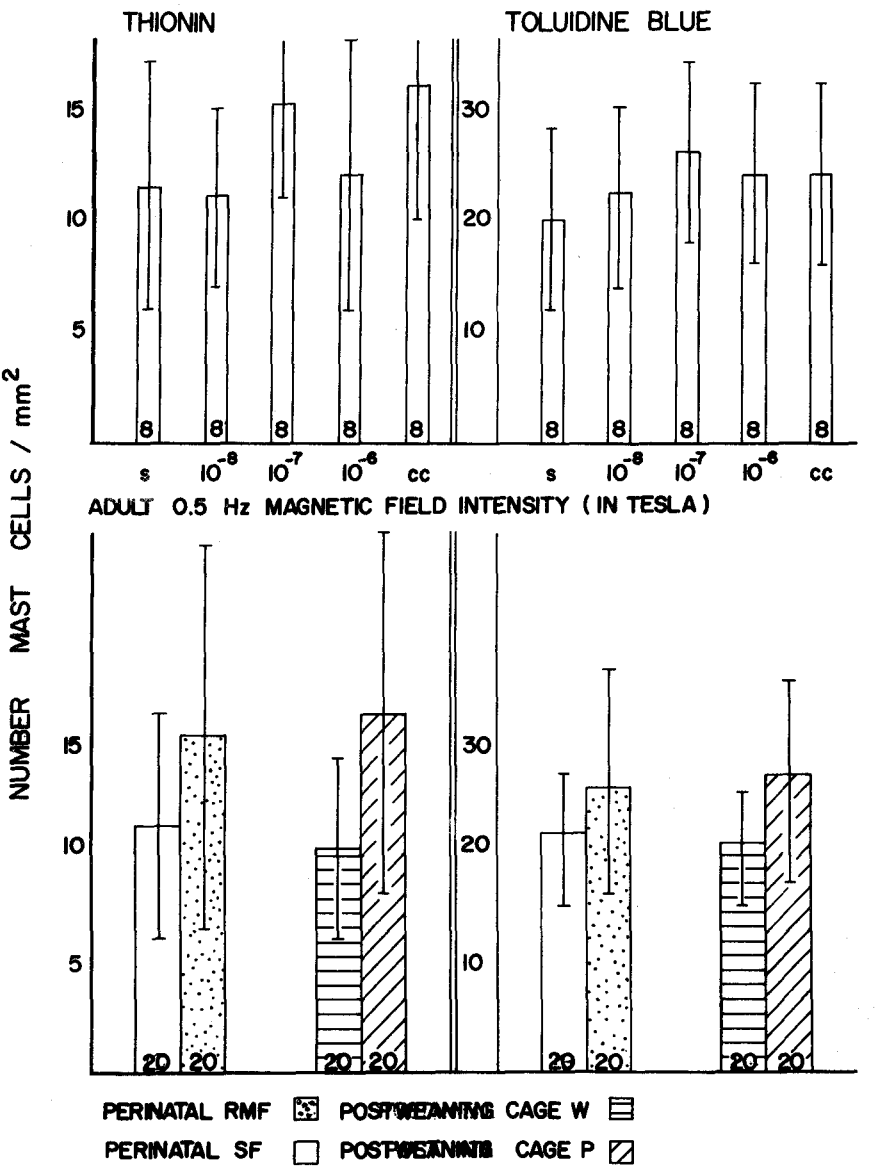

Fig. 1. Mean numbers of mast cells/mm² in thymic tissue of 40 male rats according to main effects: (1) adult exposure conditions: one of 3, 0.5 Hz magnetic field intensities, sham field (s) or colony room control (cc) conditions, (2) perinatal exposure: RMF vs sham field controls or (3) postweaning caging: plastic (P) vs wiere (W). Vertical bars indicate standard deviations. Thionin stain and toluidine blue 0 stain results are displayed on the left and right halves of the figure, respectively. Numbers in bars indicate number of subjects within each condition.

sequence, pairs of singly housed perinatally RMF- and sham field-exposed rats were exposed to each of the two intensities (10^{-8}T or sham field: 10^{-6}T or 10^{-7}T). The remaining rat, housed similarly, was maintained in the colony room as a colony control. Food and water were available ad libitum.

After 5 days, the rats were decapitated. Thymuses were removed quickly, fixed for 18 h in E.F.A. (90 parts 80% ethanol, 5 parts 30% formaldehyde, and 5 parts conc. acetic acid), dehydrated, cleared embedded in paraffin and sectioned at 6 μm. Four slides from across the central tissue depth were selected for MC staining for each rat. Two non-adjacent slides were stained with thionin after Lillie (Humason, 1972) while the other pair was stained with the quick toluidine blue 0 method. The tissue section from each slide was traversed completely at 400 X for total numbers of MCs which were defined by both metachromasia and morphology. Only: (1) MCs containing nuclei and (2) MCs in the trabeculae and primary tissue, not along the perimeters of the tissue were counted. Each slide involved between 50 and 100 visual fields. Each experimenter counted one pair of thionin and toluidine slides for each rat; the data for each slide were then converted to mean numbers of MCs/mm^2 tissue. Three way analyses of variance for the 5 adult exposure intensities (3 field, 1 sham field and 1 colony control), 2 perinatal conditions (RMF-exposed vs sham field-exposed), and 2 postnatal caging conditions (wire vs plastic) as well as additional one way analyses of variance, analyses of covariance, means, standard deviations and correlations were completed by IBM computer using SPSS programs.

RESULTS

The means and standard deviations for numbers of mast cells/mm^2 according to the main effects: 5 adult conditions (3, 0.5 Hz intensities, 1 sham field (s) and 1 colony control (cc), 2 perinatal conditions (RMF vs sham field) and 2 postweaning cage conditions (wire vs plastic) are shown in Fig. 1 for thionin and toluidine stains; there were no significant interactions although adult intensity x caging effects ($F = 2.40$; $df = 4, 20$) approached significance ($0.05 > P < 0.10$) for both stains. Three way analyses of variance for mast cells stained with thionin showed significant differences between perinatal sham vs RMF conditions ($F = 4.83$; $df = 1, 20$; $P < 0.05$) and postweaning caging conditions ($F = 13.50$; $df = 1, 20$; $P < 0.01$); all other differences were not significant ($F < 1$; $P > 0.05$). Similarly, three way analyses of variance for mast cell numbers stained with toluidine blue demonstrated significant postweaning caging differences ($F = 7.32$; $df = 1, 20$; $P = 0.01$) while the perinatal difference approached significance ($F = 3.87$; $df = 1, 20$; $P = 0.06$); again, all other F values were not significant ($F < 1$).

Since body weight differed significantly only between postweaning caging conditions (Persinger, Carrey and Lafrenière, 1978) in these studies and this variable was significant for MC numbers, covariance for body weight was performed before the analyses of variance. Thionin and toluidine stained tissue still showed significant caging ($F = 5.21$; $df = 1, 19$; $P = 0.03$ and $F = 3.70$; $P = 0.07$) and marginally significant perinatal exposure differences ($F = 3.78$; $df = 1, 19$; $P = 0.06$ and $F = 3.57$; $df = 1, 19$; $P = 0.07$). Independent one way analysis of variance demonstrated no significant effects due to adult position, order of sacrifice or perinatal breeding sequence. Mean MC numbers for thionin and toluidine stained tissue from rats perinatally exposed to 10^{-4}T, 10^{-3}T, or 10^{-6}T 0.5 Hz magnetic fields were 19.9, 19.3, 18.9 and 27.4, 25.5 and 24.9, respectively while the rats exposed to the three sham field positions averaged 17.6, 17.8 and 18.8 and 21.0, 19.8, and 22.0, respectively. There were no significant differences between perinatal intensity levels or perinatal sham-field positions. Overall correlation coefficients (r) for MC numbers for the two experimenters for thionin and toluidine stains were 0.84 and 0.76, respectively.

DISCUSSION

The thymus is a primary source of mast cells in the rat. Thymic mastocytopoesis can occur following ionizing irradiation, a variety of drug injections, immunization and testosterone and glucocorticoid injections. Cortisone, for example, initiates peritoneal MCs degranulation but promotes mast cell production in the central organs. The more destructive stimuli, such as ionizing radiation, not only increases MC numbers in the thymus but reduces its weight and size.

The results of the present study are commensurate with these established patterns. Rats that had been exposed perinatally to the RMFs displayed 20% to 35% elevations in thymic MC numbers relative to sham-field controls. These same rats (Persinger, Carrey and Lafrenière, 1978) also displayed significant 17% reductions in thymus weights and 4% increases in testicle weights; the latter effect had been reported previously (Ossenkopp, Koltek and Persinger, 1972). Although glucocorticoid and testosterone levels, factors that influence MC numbers, were not measured in these rats, the wet weight changes in the thymus and testicles imply the involvement of these chemicals.

The most impressive feature of the perinatal RMF effect, despite the large statistical overlap with sham-field controls, involves the apparent long-term expression of the change. Thymic MC differences were still evident in 200-day-old rats, despite varying postweaning treatments. Whether these differences reflect actual long-term and chronic elevations in thymic MC numbers or the enhanced tendency for perinatal RMF-exposed rats to increase thymic MC numbers following small environmental changes (e.g., removal from group cages to single cages) is not clear.

As mentioned previously (Persinger, Lafrenière and Carrey, 1978), the incorporation of a non-magnetic factor into the experimental design as a comparator variable is an important practical operation. In the present study, the perinatal RMF-associated increase in mast cell numbers was comparable in magnitude to the consequence of two types of postweaning caging treatments. One caging condition (characterized by slightly higher subject density and accessible bedding) was associated with significant 11% elevations in gross body weight, absolute testicle weights and changes in a variety of blood measures (unpublished data) relative to the second caging condition (suspended (11 mm) wire mesh cages); the former rats had elevated MC numbers. However unlike the caging effects, the perinatal RMF-exposed rats displayed no gross changes in body weight but significant changes in both relative and absolute thymus and testicle weights as well as significant decreases in serum glutamate oxaloacetic transaminase activity, lactate dehydrogenase activity and blood urea nitrogen levels (unpublished data).

Consequences of adult exposure to low intensity 0.5 Hz magnetic fields, within the range of natural atmospheric values ($< 10^{-9}$T to 10^{-6}T) were not statistically evident in thymic MC numbers. Furthermore, the environmental differences between the continuous lighting and masking noise of the experimental room and the 12L:12D and variable noise of the colony room were not sufficient to significantly alter MC numbers. It is also apparent that perinatal exposures to RMF-fields at intensities sufficient to be correlated with later behavioral changes (Persinger, 1969; Persinger and Pear, 1972) do not appear to alter adult susceptibility to lower intensity 0.5 Hz fields since no adult intensity by perinatal condition interactions existed in the present study.

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